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Physiological Changes in *Portulacaria afra* (L.) Jacq. during a Summer Drought and Rewatering[1]

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ABSTRACT

The changes of titratable acidity, enzyme activity, water status, and pigment composition were studied in *Portulacaria afra* (L.) Jacq. during a normal summer drought and rewatering. Two groups of plants were grown outside under a clear plastic canopy with water stress initiated at 2-week intervals in May 1986. Drought resulted in a linear decrease of fresh weight for 80 days and there was no further fresh weight change for the next 65 days. Nocturnal CO_2 uptake remained measurable for 83 days. Cessation of exogenous CO_2 uptake corresponded to the point where the pressure potential (ψ_p) became zero. Ribulose-1, 5-bisphosphate (RuBP) and phosphoenolpyruvate carboxylase were reduced to 50% of this activity by the end of the drought period. Phosphoenolpyruvate carboxykinase activity was undetectable after 120 to 140 days of drought. Chlorophyll (Chl) levels decreased with a preferential loss of Chl *a* over Chl *b*. Carotenoid content was relatively constant over the course of the drought period. After 145 days of drought, plants responded to rewatering within 24 hours; ψ_p became positive and daytime CO_2 uptake resumed after 24 hours. After 3 days, RuBP carboxylase activity reached control levels. Activity of the CAM pathway recovered after 5 days, as noted by increased diurnal acid fluctuations. Phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase activity fully recovered within 6 days. Chl levels were greater than control levels within 5 days. Chl *a*/*b* ratios took 27 days to return to control levels. The results indicated that *P. afra* can withstand a normal summer drought by utilizing the CAM and CAM-idling pathway for 130 to 140 days. The plants respond rapidly to rewatering because of the conservation of enzyme activity and the quick recovery of ψ_p.

There have been relatively few studies of drought on CAM species (15, 24) considering that CAM is an adaptation to arid or semiarid environments. A number of papers with facultative CAM species have reported on the induction of CAM photosynthesis from C_3 photosynthesis during water stress (2, 8, 19, 21–23, 29, 30). Other researchers have studied constitutive CAM species to ascertain the effects of water stress on the CAM pathway during the shift from CAM to CAM-idling (17, 20, 27). CAM-idling is a response to drought in which stomata close, restricting water loss and exogenous CO_2 uptake. By recycling organic acids, the plants maintain metabolic activity and consequently respond rapidly to rainfall (24).

Short-term water stress (2–4 weeks) of facultative CAM plants resulted in increased diurnal organic acid fluctuations, increased nocturnal CO_2 uptake and PEP[3] carboxylase activity, with concomitant decreases in daytime CO_2 uptake, and tissue water potentials (8, 19, 23, 24). Long-term imposition of drought reduced the gas exchange and acid fluctuation to zero, and led to a shift to less negative (more CAM-like) carbon isotope composition (6, 20, 22, 27).

Few studies have investigated the simultaneous changes that occur in enzyme activity and pigment composition during the shift to CAM-idling under long-term water stress in small leaf succulents. In *Sedum pulchellum*, researchers followed PEP carboxylase and RuBP carboxylase activity for 81 d during CAM induction and found only a small increase in PEP carboxylase activity and a decline in RuBP carboxylase activity for the low and high water stress treatment (19). After 1 month of water stress, *Xerosicyos danguyi* showed no differences in PEP carboxylase and NADP-malic enzyme activity (17). Water stress of *Sedum sexangulare* results in higher Chl *a*/*b* ratios and β-carotene content, but lower lutein and neoxanthin contents (25, 26).

Portulacaria afra is a perennial, small-leaf succulent, endemic to the Mediterranean climates of South Africa and shifts from C_3 to CAM photosynthesis when grown outside during the summer despite irrigation (5). We have previously examined the seasonal response to drought and rewatering of the shift to CAM (or CAM-idling) by following the change in gas exchange and titratable acidity characteristics (6). The purpose of this investigation was to study the utilization of the CAM and CAM-idling pathway during a summer drought and rewatering by following the changes in enzyme activity, pigment composition, and gas exchange.

MATERIALS AND METHODS

Plant Material. *Portulacaria afra* (L.) Jacq. clones were propagated from a large shrub growing outdoors on the UC Riverside campus. Cuttings were rooted in UC-2 potting mix (loamy sand). All plants were grown outside under natural light, photoperiods, and temperatures characteristic for Riverside (see Ref. 6 for a description). Irrigated plants grown in either 1 or 2 gallon pots were watered every 3rd d to maintain high tissue-water potentials. The plants were fertilized bimonthly. Two groups of three plants grown in 5-gallon pots were water stressed at a 2-week interval starting May 5, 1986. The water-stressed groups were grown under a clear plastic canopy to ensure that no rainwater entered the soil. Water was withheld until September 26 for the first group and until October 16 for the second group. Plants were rewatered by filling the pots with water and spraying the leaves with water.

Titratable Acidity. Nine to 12 leaves were collected in the

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[3] Abbreviations: PEP, phosphoenolpyruvate; RuBP, ribulose 1,5-bisphosphate; ψ_w, water potential; ψ_π, osmotic potential; ψ_p, pressure potential.

morning and afternoon. Samples were frozen (−20°C) until assayed. Leaf punches of 0.24 cm^2 were weighed, ground in glass-distilled water with a tissue grinder, and titrated with 0.01 N KOH to a pH 7.0 endpoint. Results are expressed as μeq cm^{-2} ± SE.

Gas Exchange Studies. CO_2 uptake was measured using a dual isotope porometer as previously described by Guralnick and Ting (6). Gas exchange measurements of water stressed plants were taken predawn for the nighttime measurements (phase I; see Ref. 18 for a description of the four phases of gas exchange of CAM plants) and early morning (phase II) to monitor the decrease in gas exchange for the plants. It was found previously that these were the last two phases of CO_2 uptake that could be measured during water stress (6). Measurements were taken at approximately 1-month intervals until exogenous CO_2 uptake was zero. Data are expressed as μmol m^{-2} s^{-1} ± SE.

Water Potential. Water potential (ψ_w) was measured predawn and midday (12:00–1:00 PM) using a pressure bomb (Plant Water Status Console, Soil moisture Co., Santa Barbara, CA) on branches with leaves attached. Mature leaves were harvested and frozen for subsequent measurements of osmotic potential (ψ_π). Two leaves per sample were thawed in a 24°C water bath for 20 min. The cell sap was extracted using a french press and placed into a vapor pressure osmometer (Wescor Inc., Logan, UT). The osmometer was calibrated using known sucrose solutions. These two measurements allowed a pressure potential (ψ_p) to be estimated by utilizing the equation (14):

$$\psi_w = \psi_\pi + \psi_p$$

Although calculations occasionally resulted in negative ψ_p, we assumed that these were in error and are reported as 0. Data are expressed as MPa ± SE.

Enzyme Assays. Leaf samples for PEP carboxylase and PEP carboxykinase were collected in the afternoon. The tissue (1 g fresh weight for the control plants and 0.5 to 1.0 g fresh weight for the water-stressed plants) was ground in a tissue grinder in 10 ml of extraction buffer at 4°C containing 100 mM Hepes-KOH, 10 mM $MgCl_2$, 10 mM DTT, Triton X-100 (1% v/v), and 1% PVP-40 all adjusted to pH 7.8. The sample was centrifuged at 4°C at 10,000 rpm for 15 min. The crude extract was used for enzyme assays. The extraction medium for RuBP carboxylase was the same except that 10 mM $NaHCO_3$, 10 mM Na ascorbate, and 0.5% BSA was added.

PEP carboxylase and PEP carboxykinase were assayed spectrophotometrically by following the oxidation of NADH at 340 nm at 27°C. The PEP carboxylase assay mixture contained 100 mM Hepes-KOH (pH 7.8), 10 mM $MgCl_2$, 1 mM $NaHCO_3$, 0.2 mM NADH, 5 IU malate dehydrogenase, 3 mM PEP, and 200 μl crude extract in a total volume of 3 ml. The PEP carboxykinase assay mixture was modified from Leegood and Ap Rees (11) and contained 100 mM Mes-KOH (pH 6.0), 2.5 mM ADP, 5 mM $MnCl_2$, 50 mM $KHCO_3$, 0.35 mM NADH, 5 IU malate dehydrogenase, and 3 mM PEP. The reaction was assayed at pH 6.0 to minimize interference from PEP carboxylase (4) and was found to be ADP and Mn dependent. In addition, PEP carboxykinase was assayed by radiolabel techniques utilizing either the ATP-dependent exchange reaction or the ADP-dependent carboxylation (4).

RuBP carboxylase was assayed according to the method of Lorimer *et al.* (12). Leaves were harvested, weighed, and ground as quickly as possible to obtain a fresh extract. The assay mixture contained 50 mM Tes-Bicine (pH 8.1), 10 mM $MgCl_2$, 10 mM DTT, 20 mM $NaHCO_3$ (with $NaH^{14}CO_3$ at a specific radioactivity of 0.50 μCi/μmol), and 100 μl crude extract. RuBP (2.5 mM) was added to initiate the reaction. The reaction was stopped after 1 min by adding 6 N HCl. The samples were slowly air dried to remove the excess $^{14}CO_2$. After drying, 0.5 ml of H_2O and 10 ml Betaphase scintillation cocktail (Westchem Co.) were added and then counted by liquid scintillation methods.

All results are expressed as μmol m^{-2} s^{-1} ± SE.

Pigment Determination. Leaf discs of 0.24 cm^2 taken from samples for enzyme determination were used to estimate Chl and carotenoid amounts. The samples were ground in 100% acetone and brought to 5 ml volume. The samples were centrifuged for 10 min at 2500 rpm. Equations of Wellburn and Litchtenthaler (28) were utilized to estimate Chl and carotenoid concentration. Samples were read at 730 nm for particulate matter concentration and were subtracted from the other readings (18). Data are expressed as μg cm^{-2} ± SE.

RESULTS

Drought Characteristics. Drought resulted in a linear disease in leaf fresh weight per area for approximately 60 to 80 d after which only slight fluctuations in the leaf fresh weight occurred (data not shown). The water-stressed leaves were approximately 25% of the control fresh weight at the end of the drought, which corresponded to a 30% relative water content. At drought initiation, day-night fluctuation of organic acids of 18 to 30 μeq cm^{-2} were observed, after which the acid fluctuations slowly decreased (Fig. 1). After 80 d, there were very small acid fluctuations of 3 to 5 μeq cm^{-2}. The control plants, without drought treatment, showed an increase in the day-night organic acid fluctuation at the start of the experiment indicating increased CAM activity. By the end of the experiment, the control plants were shifting back to the C_3 pathway of photosynthesis as indicated by decreased acid fluctuations. The leaf fresh weight and titratable acidity data indicated both stressed groups showed similar changes during drought and, thus, are grouped together in the remainder of the "Results" section.

Gas Exchange. Phase I (nocturnal CO_2 uptake) and phase II (early morning daylight uptake) CO_2 uptake was monitored during the course of the experiment. After approximately 41 ± 7 d without water, phase I uptake was 35 ± 5% (0.92 ± 0.13 μmol m^{-2} s^{-1}) of the control plants and phase II uptake was 11 ± 2% (0.50 ± 0.10 μmol m^{-2} s^{-1}) of the control. At d 69, phase I uptake remained at 30 ± 7% of the control group and phase II uptake was reduced to 8 ± 3% of the control group. By d 83, no phase II uptake was measurable and phase I uptake had been reduced in half to 17 ± 6% of the control plants. After 103 d without water, no exogenous CO_2 uptake could be measured. Thus, daytime and nighttime CO_2 uptake remained measurable up to 69 ± 7 or 83 ± 7 d, respectively, after the last watering.

Water Potential. Predawn and midday ψ_w decreased during the course of the drought. After 41 ± 7 d, predawn potentials were −0.61 ± 0.08 MPa for the water stress plants compared to −0.17 ± 0.05 MPa for control plants. Midday ψ_w of the water stressed and control plants were reduced to −1.0 ± 0.18 MPa.

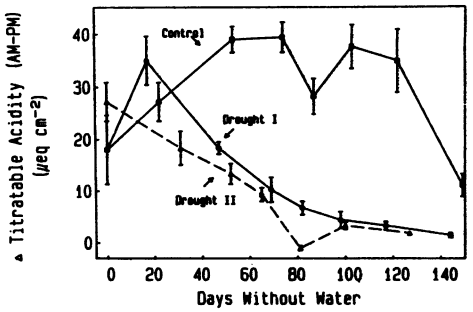

FIG. 1. Time course of diurnal changes in titratable acidity (AM-PM). Water stress initiated on May 5, 1986 (drought I, ●——●) and May 19, 1986 (drought II, ▲- - -▲; control, ■——■). Error bars represent 1 SE of the mean when large enough for all figures.

While ψ_π in the water stressed plants was at -1.15 ± 0.07 MPa compared -0.69 ± 0.07 MPa for the control plants and showed no change from predawn to midday. These results indicated measurable ψ_p of 0.2 to 0.5 MPa in the water-stressed plants. However, after 103 d without water, the water-stressed plants had a ψ_w of -2.32 ± 0.12 MPa and a ψ_π of -1.92 ± 0.18 MPa, the irrigated plants showed no change. These results gave an estimate of negative ψ_p (-0.4 MPa) for the water-stressed plants. Problems of expressing sap from the water-stressed leaves may have resulted in an underestimate of ψ_π, thus causing a calculated negative ψ_p. We assumed that ψ_p was 0.

Enzyme Activity. PEP carboxylase activity increased at the beginning of the drought, and corresponded to the increased acid fluctuations (Figs. 1 and 2). After 40 d of drought, the activity had decreased to 50% of the control level. The decarboxylase, PEP carboxykinase, decreased slowly in activity becoming undetectable after 120 d while activity in the control plants remained high (15–20 μmol m^{-2} s^{-1}). RuBP carboxylase activity showed 55% reduction at the beginning of the drought period, but remained relatively constant thereafter.

Pigment Composition. Total Chl decreased slowly during the drought from 30 to 24 μg cm^{-2} (Fig. 3). However, the decrease up to 90 d was similar to the irrigated plants. After 90 d, the water-stressed plants showed a decline when compared to the irrigated plants. The Chl *a*/*b* ratio decreased in both the water-stressed and the control plants. The water-stressed plants showed a larger decrease reaching a Chl *a*/*b* ratio of 3.40 (Fig. 4, inset). Control Chl *a*/*b* ratios remained at approximately 4.50 during the course of the summer and rose to over 5.00 in October (data not shown). In addition, the decrease in the Chl *a*/*b* ratio for the water-stressed plants was due to a decrease in Chl *a*, while Chl *b* levels remained relatively constant throughout the drought (Fig. 4). The control plants showed a decrease in both Chl *a* and *b*, but a greater decrease in Chl *a* which accounted for the lower Chl *a*/*b* ratio (data not shown).

In contrast, the cartenoid content remained at 200 to 210 μg cm^{-2} during the course of the drought and was similar to the control plant levels (Fig. 4).

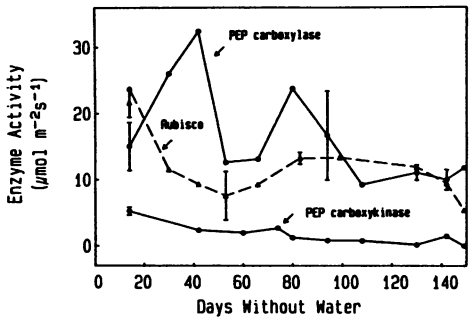

FIG. 2. Time course of PEP carboxylase (●——●), RuBP carboxylase (▲- - -▲), and PEP carboxykinase (○——○).

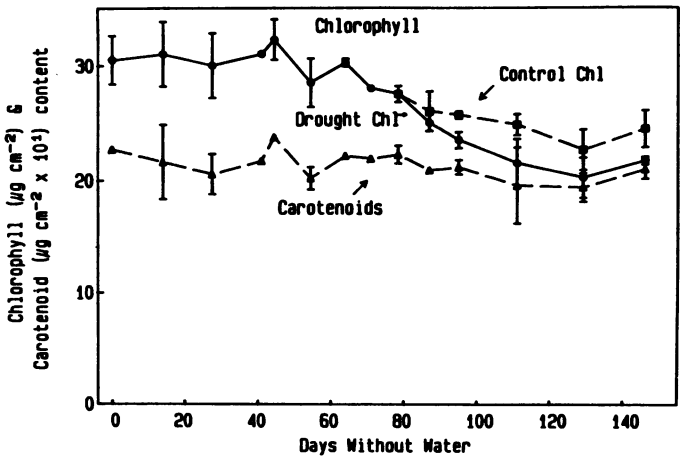

FIG. 3. Time course of changes in Chl, drought (●——●) and control (■- - -■) and carotenoid (control and drought ▲- - -▲) composition.

RECOVERY

Gas Exchange and Titratable Activity. Daytime CO_2 uptake rates recovered to 25% of the control levels (data not shown) within 24 h after rewatering; however, no nocturnal CO_2 uptake was measured. After 72 h, daytime gas exchange was 50% of the control plants, and there was some measurable nocturnal CO_2 uptake and acidification (Fig. 5). Small acid fluctuations were noted by d 2 and 3. By d 5, a diurnal acid fluctuation of 27 μeq em^{-2} was measured in the rewatered plants and was larger in magnitude than the control plants (11 μeq cm^{-2}), which were shifting back to the C_3 pathway (5).

Water Relations. Predawn and midday water potential measurements showed similar characteristics during recovery (Fig. 6, A and B). After 24 h, ψ_w and ψ_π had not fully recovered and were still lower than the control plants; ψ_p had become slightly positive. By 48 h, the water relations of the recovered plants was not significantly different from the control plants, as indicated by the ψ_w and ψ_p. Osmotic potential was significantly higher in the

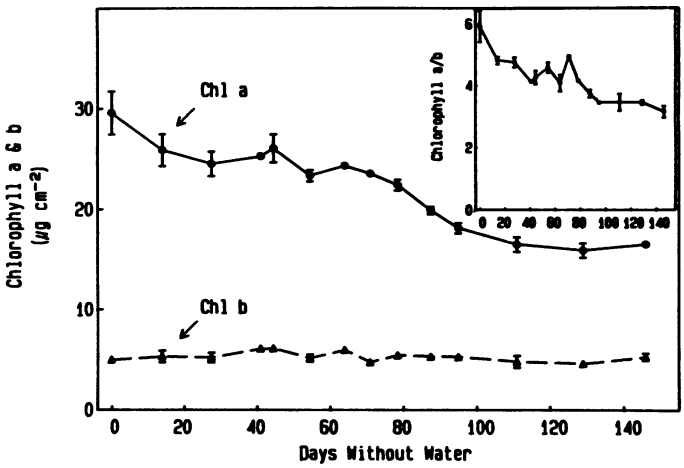

FIG. 4. Time course of Chl *a* (●——●) and Chl *b* (Δ- - -Δ) composition. Inset, Chl *a*/*b* ratios over the course of the drought; X-axis same as in large figure.

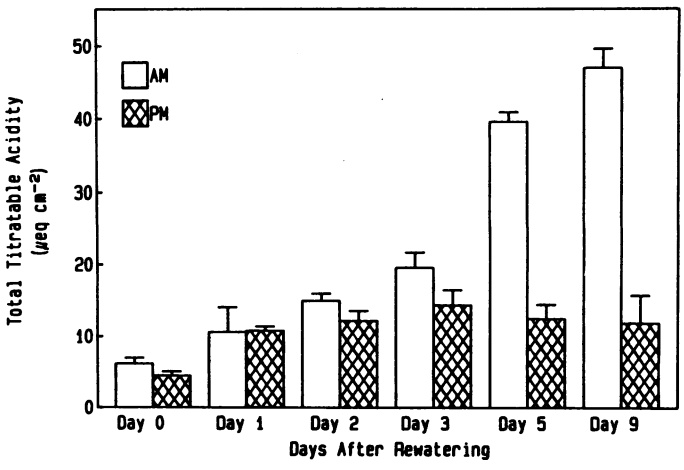

FIG. 5. Change in titratable acidity during rewatering.

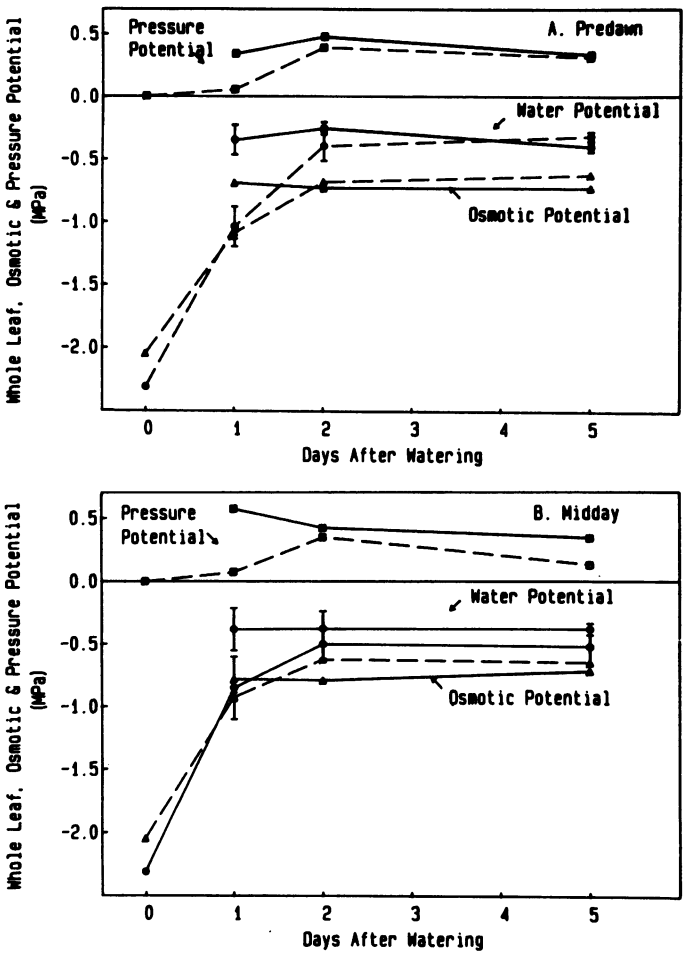

FIG. 6. A, Changes in predawn water potential; B, changes in midday water potential during rewatering. ψ_w (control, ●——●; rewatered, ○- - -○); ψ_π (control, ▲——▲; rewatered, △- - -△); and ψ_p (control, ■——■; rewatered, □- - -□).

Table I. *Recovery of Enzyme Activity in P. afra after Rewatering*

Time after Rewatering	PEP Carboxylase	PEP Carboxykinase	RuBP Carboxylase
d		*μmol m⁻² s⁻¹*	
Control	22.72 ± 3.7	14.87 ± 2.3	19.75 ± 1.0
0	11.78 ± 0.4	ND[a]	8.88 ± 1.9
1	7.77 ± 1.0	ND	7.64 ± 1.9
2	9.67 ± 0.7	1.50 ± 1.0	6.74 ± 1.5
3	—[b]	—	16.58 ± 0.9
6	21.97 ± 1.7	12.25 ± 2.7	—

[a] Not detectable, rates are equal to background PEP carboxylase activity. [b] Not determined on that date.

recovered plants compared to the control.

Enzyme Activity. RuBP carboxylase activity showed slight increases during the first 48 h after rewatering but by 74 h, the activity increased to control plant levels (Table I). The enzymes of the CAM pathway showed different types of recovery. PEP carboxylase showed a slight decrease in activity for the first 48 h, but activity was fully recovered by the sixth day. In contrast, PEP carboxykinase activity was not detected after 24 h and was very low after 48 h. After 6 d, the activity had increased to control levels.

Pigment Composition. Recovery of Chl showed a slight decrease from the water-stressed levels for the first 48 h (Table II). Chl levels increased to higher than control levels by d 5, and

Table II. *Recovery of Chl and Carotenoids in P. afra after Rewatering*

Time after Rewatering	Chl	Chl *a/b*[a]	Carotenoids
d	*μg cm⁻²*	*ratio*	*μg cm⁻²*
Control	25 ± 2	5.08 ± 0.06	183 ± 8
0	23 ± 0	3.41 ± 0.14	209 ± 3
1	21 ± 2	3.43 ± 0.31	184 ± 22
2	20 ± 0.5	3.36 ± 0.06	189 ± 2
5	29 ± 1	3.99 ± 0.32	207 ± 19
10	29 ± 2	4.45 ± 0.17	221 ± 18
15	36 ± 2	4.38 ± 0.07	246 ± 12
27	41	5.07	277

[a] Chl *a/b* ratios were determined on *Kalanchoe* spp. (2.96), *Portulaca oleracea* (2.54), and *Ananus comosus* (3.42) to check the assay procedure.

increased to 41 μg cm⁻² by d 7. The Chl *a/b* ratio showed a slower recovery and did not return to control levels until 27 d after rewatering. This was due to the faster recovery of Chl *b*. Carotenoid content increased similarly to Chl content and showed a 50% increase over control carotenoid levels.

DISCUSSION

The results indicated that *Portulacaria afra* during a summer drought showed differential expression of CAM activity. The first 20 d of the drought showed slight increases in titratable acidity and PEP carboxylase activity above that of the control plants. The increased CAM activity observed initially during drought is similar to previously reported research (8, 23). Initially, there was also a large reduction in daytime CO_2 uptake which indicated greater sensitivity of water stress as has been shown previously (8). However, RuBP carboxylase activity was less sensitive to long-term drought and retained 50% of the control activity which is similar but less than the resurrection plant *Selaginella lepidophylla* (9). The second phase of drought (20–80 d) showed a slow decline in CAM and C_3 activity, where exogenous gas exchange was reduced and the acid fluctuation was primarily due to recycling of respiratory CO_2. The third phase (80–140 d) was characterized by a total absence of day and nighttime CO_2 uptake and a minimal acid fluctuation. Gas exchange was measurable until the 83rd d after watering and was similar to previous reports by Guralnick and Ting (6). The absence of CO_2 uptake and a small acid fluctuation indicated very little CAM-idling metabolism as shown by a nearly zero (3 μeq cm⁻²) acid fluctuation when compared to a 78 μeq cm⁻² for *Opuntia basilaris* after a 6 month water stress (16). These results were similar in character to *Sedum rubrotinctum* grown under greenhouse conditions (22). However, *P. afra* reached much lower leaf water, osmotic, and pressure potentials in a shorter time possibly because of being grown outside under natural light and temperatures.

The changes in CAM activity corresponded to the observed enzyme activities. The primary enzymes of the CAM pathway, PEP carboxylase and PEP carboxykinase, were reduced in activity. After 140 d of drought, PEP carboxylase still retained approximately 50% of the control activity. In contrast, PEP carboxykinase activity became undetectable and since PEP carboxykinase is the primary decarboxylase in *P. afra*, the CAM-idling mechanism would be markedly reduced in activity as was noted by the titratable acidity data. In stem succulents, the CAM-idling mechanism is thought to maintain metabolic activity until the next rainfall (16, 20). However, it is becoming increasingly clear that the CAM-idling mechanism will aid in short-term drought, but not in a longer drought (6 months or longer) for small-leaf succulents (22, 27). This may be due to water potentials becoming increasingly negative for *P. afra* (−2.30 to −3.30 MPa) when compared to stem succulents and

cacti (−1.00 to −1.50 MPa) (6, 10, 16, 20). Also, it may be due to the fact that the decarboxylase appeared to be more sensitive to drought than PEP carboxylase, and may be a limiting factor in the CAM-idling metabolism. This has been observed in the NADP-malic enzyme plant, *Opuntia ficus-indica* (3).

Pigment composition did not change similarly to the CAM activity during the first part of the drought. The decrease in total Chl was a seasonal change probably due to the increasing amount of light during the summer. Levels of carotenoids remained high in both groups of plants possibly protecting the leaf photosynthetic apparatus during the summer.

During the latter part of the drought, between the 80th and 100th d, total Chl of the water-stressed plants was reduced when compared to that of the control plants. The reduction in total Chl was due to a drop in Chl *a*, while Chl *b* levels remained constant throughout the drought. This differs from the results obtained for *Sedum sexangulare* which showed a decrease in Chl *b* during drought and was thought to be related to changes in the light-harvesting-complex proteins (25, 26). The photosynthetic apparatus of *O. basilaris* undergoes photoinhibition when nocturnal acid accumulation is minimal and the CAM-idling metabolism is reduced in activity (1). Thus, the observed changes in *P. afra* may be due to preferential photooxidation or metabolism of Chl *a* under photoinhibitory conditions when the CAM-idling mechanism is reduced in activity. This is supported by the fact that the CAM-idling metabolism was reduced in function as noted by decreased acid fluctuation and reduced enzyme activity. This response is similar to three different *Opuntia* spp. which showed Chl loss after the cessation of the CAM-idling metabolism (10).

Daytime CO_2 uptake recovery after 24 h corresponds to the recovery of pressure potential in the rewatered plants. The recovery of daytime CO_2 uptake was aided by the 50% conservation of the RuBP carboxylase activity in the water-stressed plants. In addition, other enzymes of the Calvin cycle are highly conserved in *S. lepidophylla* (9), so it may by presumed that they are also conserved in this species as well, thus accounting for the quick resumption of daytime CO_2 uptake upon rewatering.

Recovery of nocturnal CO_2 uptake was slower than daytime CO_2 uptake and required 72 h. The resumption of CAM activity, as indicated by a large fluctuation of titratable acids, was not complete until the 5th d, and corresponded with the return of full PEP carboxykinase activity. Thus, the CAM activity recovered faster than was previously found, but *P. afra* evidently does not recover CAM activity as quickly as *O. basilaris*, a stem succulent (6, 20).

It has been hypothesized that the resumption of CAM activity is correlated with water relation parameters (13, 20, 27). It has previously been suggested that malate efflux from the vacuole is related to turgor pressure, while influx to the vacuole is turgor independent (13). However, the data gathered here indicated that water-relation parameters including pressure potential returned to control plant levels 48 h after rewatering, while resumption of CAM activity required 5 d. Therefore, the above hypothesis may be incorrect for facultative CAM species. Thus, other factors must be limiting the return of CAM activity in *P. afra*, such as enzyme synthesis (PEP carboxykinase) or perhaps energy, since influx of malate into the vacuole is ATP dependent (13).

Pigment compositional changes during recovery were also correlated with the resumption of CAM activity, but this may be an independent phenomenon. Two d after rewatering, Chl and carotenoid levels remained at drought levels. By the 5th d, Chl and carotenoids increased to levels greater than control plants levels, while the Chl *a*/*b* ratio took 27 d to return to control plant levels. This may be related to the lower light levels in October during rewatering and the slower seasonal recovery of pigment levels of the control plants. The Chl *a*/*b* ratio in *P. afra* took 27 d to return to control plant levels. Further research is needed to ascertain which components of the Chl proteins are recovering during the first days of rewatering.

This report indicates that *P. afra* does withstand long-term summer drought by utilizing the CAM and CAM-idling metabolism. *P. afra* retains more than just the apical leaves as *S. rubrotinctum* does after long-term drought (7, 22). After 9 months, the water-stressed *P. afra* plants still retained their leaves. Physiological changes associated with long-term stress indicate that daytime CO_2 uptake is more sensitive to water stress than nocturnal CO_2 uptake. However, the conservation of RuBP carboxylase activity results in a rapid response to rewatering and resumption of daytime CO_2 uptake over nighttime uptake. The CAM-idling mechanism, which is thought to maintain metabolic activity and allow a quick resumption of gas exchange (24), operated in *P. afra* for approximately 50 d after exogenous CO_2 uptake ceased. Earlier research indicated that the CAM-idling metabolism can function for longer periods depending on the season when drought is initiated (6). However, in *Opuntia* spp., CAM-idling may operate for longer periods of drought and is probably related to the greater amounts of water that can be stored in the stem as compared to the small amount of water in the leaves of *P. afra* (16, 20). Further research is needed to clarify the mechanism that allows *P. afra* leaves to remain on the plant in full sunlight under conditions that should cause photooxidation. In addition, a more detailed study of the rapid recovery of *P. afra* plants and the C_3 pathway after long-term drought is required. It is becoming increasingly clear that the CAM/CAM-idling metabolism may only partially explain the survival of the plant during long-term drought.

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